

Carbon storage as affected by different site preparation techniques two years after mixed forest stand installation

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Abstract

Aim of study: This study aims at evaluating the impact of site preparation techniques prior to plantation on carbon storage and distribution in a young mixed stand of *Pseudotsuga menziesii* (PM) and *Castanea sativa* (CS).

Area of study: The experimental field was established near Macedo de Cavaleiros, Northern Portugal, at 700 m elevation, mean annual temperature 12°C and mean annual rainfall 678 mm.

Material and methods: The experimental layout includes three replicates, where the different treatments corresponding to different tillage intensities were randomly distributed (high, moderate and slight intensity), in plots with an area of 375 m² each. Twenty six months after forest stand installation, samples of herbaceous vegetation (0.49 m² quadrat), forest species (8 PM and 8 CS) and mineral soil (at 0-5, 5-15, 15-30 and 30-60 cm depth) were collected in 15 randomly selected points in each treatment, processed in laboratory and analyzed for carbon by elemental carbon analyzer.

Main results: The results obtained showed that: (i) more than 90% of the total carbon stored in the system is located in the soil, increasing in depth with tillage intensity; (ii) the contribution of herbaceous vegetation and related roots to the carbon storage is very low; (iii) the amount of carbon per tree is higher in CS than in PM; (iv) the global carbon storage was affected by soil tillage generally decreasing with the increase of tillage intensity. Accordingly, carbon storage capacity as affected by the application of different site preparation techniques should be a decision support tool in afforestation schemes.

Key words: site preparation; forest species; herbaceous vegetation; carbon storage; mineral soil; Portugal.

Introduction

Mechanical site preparation to install forest plantations can be justified by numerous reasons as limiting weed competition, increasing effective soil depth, reducing soil strength to encourage root expansion, improving water holding capacity and nutrient availability. These effects are especially important in the Mediterranean region, where water shortage is the main factor limiting the success of afforestation (Daget, 1977; Ojasvi *et al.*, 1999; Kanegae *et al.*, 2000; Silva, 2002; Fonseca *et al.*, 2011). The correct installation of forest stands, which results in better productivity and lower impacts on soil and environment has also to consider the growing concern with effects on global warming and climate change (Birdsey *et al.*, 1993). In this context, it is essential to select appropriate site

preparation techniques for new plantations, in order to satisfy an increasingly current requirement in sustainable resources management (Worrell and Hampson, 1997; Zheng *et al.*, 2008).

Caspersen *et al.* (2000) showed that land-use change is the main factor governing the rate of carbon storage in terrestrial ecosystems in the USA. Following afforestation, changes inevitably happen in the quantity, quality, and spatial distribution of soil carbon. Mechanical disturbance lead to increased organic matter decomposition, because it breaks up organomineral aggregates and exposes to decomposers (Turner and Lambert, 2000; Balesdent *et al.*, 2000; Madeira *et al.*, 2002; Schulp *et al.*, 2008; Zheng *et al.*, 2008), which is site-specific and varies with system's disturbance intensity (Post and Kwon, 2000; Fonseca, 2005). There is a wide variation in the time period and the rate at which carbon may accumulate in soil, depending on site preparation, plant productivity, soil characteristics, past history of carbon gains, climate, and site management (Post and

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Kwon, 2000; Paul *et al.*, 2002). Probably, all of the forests in Mediterranean region have been transformed by humans to some degree at some time in the past. Nevertheless, little is known about the effects of site preparation for afforestation on changes in carbon pool under Mediterranean climate. This knowledge may help guiding the choice of soil preparation techniques for sustainable systems establishment, specifically those aimed at carbon accumulation. Forest trees and forest soils have both a huge capacity to accumulation and release carbon (Dixon *et al.*, 1994; Mendham *et al.*, 2003; Jacobs *et al.*, 2009). Forest trees contribute differently to carbon storage in forest ecosystems depending on the state of stand development (age) and on forest species silvicultural characteristics (Silver *et al.*, 2000; Turner and Lambert, 2000). Soil carbon is a very significant compartment of total carbon storage in forest ecosystems, but has received less attention than carbon in tree biomass (Johnson, 1992; Harrison *et al.*, 1995; Turner and Lambert, 2000).

The present study aims to evaluate carbon storage and distribution on a mixed forest stands recently established under different site preparation techniques. Total carbon inventories in system include carbon stored in forest species, herbaceous vegetation and mineral soil.

Material and methods

The experimental field was established near Macedo de Cavaleiros, Northeast Portugal at 41° 35' N and 6°

57' W, 660 to 701 m altitude, mean annual temperature 12°C, mean annual rainfall 678 mm, with a typically Mediterranean seasonal distribution (INMG, 1991).

The experimental protocol consisted of six treatments corresponding to different intensities of soil disturbance, installed in plots with 375 m² (25 m × 15 m in size), each were randomly distributed in three blocks, that replicate the set of treatments tested. *Pseudotsuga menziesii* (PM) and *Castanea sativa* (CS) were used as forest species, on 4 m × 2 m density and alternate rows (2 for PM, 2 for CS), summing 12 plants per row in each plot (1,280 trees ha⁻¹). The mixed stand combines native and exotic species. *Castanea sativa* is native in Portugal, with particular interest in the region where this study took place, for fruit and wood production. The exotic species *Pseudotsuga menziesii* was introduced in Portugal in 1846 and widely used since then in many afforestation projects, due to its high potential for timber production, particularly expressed in mountain silviculture (Luis and Monteiro, 1998). The treatments, representing different soil disturbance intensities were selected among a set of commonly applied in afforestation schemes (Table 1). Prior to site preparation operations a heavy disc harrowing was performed in the area, in order to reduce or eliminate existing shrub vegetation. Experimental field includes also a treatment without disturbance (TSMO) that corresponds to the original soil and is taken as a reference for comparison with the remainder treatments in what concerns the tillage effects on soil carbon storage. This treatment was not planted. More details, about experimental field, can be found in Fonseca *et al.* (2011).

Table 1. Treatments tested in the experiment, related to six site preparation techniques

Intensity of soil disturbance	Treatment	Description of site preparation techniques, explaining treatment code (depth of soil disturbance)
No disturbance	TSMO	Control treatment, T, no intervention, SMO (no soil disturbance on the original abandoned field)
Slight disturbance	SMPC	No subsoiling, no ploughing, SM, plantation with hole digger, PC (60 cm depth)
	RCAV	Subsoiling over the whole area, RC, with covering shovel, AV (around 60 cm depth)
Moderate disturbance	SRVC	No subsoiling, SR, contour bunds shaped by two plough passes, VC (around 90 cm depth on contour strips)
	RLVC	Subsoiling in future plantation rows, RL, contour bunds shaped by two plough passes, VC (around 90 cm depth on contour strips)
High disturbance	RCVC	Subsoiling over the whole area, RC contour bunds shaped by two plough passes, VC (around 90 cm depth on contour strips)
	RCLC	Subsoiling over the whole area, RC, contour ploughing over the whole area, LC (around 90 cm depth)

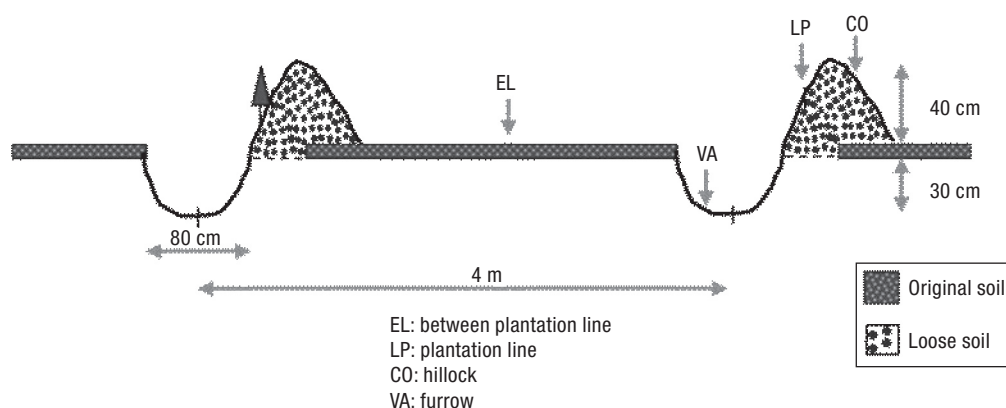


Figure 1. Scheme representing furrow hillock site preparation techniques applied in the experiment (SRVC, RLVC and RCVC).

The implementation of site preparation techniques contributed for different percentages of soil disturbance in plots area. The slight site preparation caused soil disturbance among 10-14% and 22-25% in SMPC and RCAV, respectively. In the moderate soil tillage intensity, figures were around 49-52% (SRVC and RLVC) and for the intensive soil tillage ranged between 70-75% in RCVC and 95-100% in RCLC. Accordingly, it was considered that the plots of treatments TSMO (control), SMPC, RCAV and RCLC presented individually, features more or less homogeneous throughout the plot, while the plots of treatments SRVC, RLVC and RCVC, due to application of furrow hillock created soil strips with different intensity of soil disturbance, as illustrated in Fig. 1.

Twenty six months after forest stand installation, samples of herbaceous vegetation, forest species and mineral soil were collected in all treatments (TSMO, SMPC, RCAV, SRVC, RLVC, RCVC and RCLC). Soil samples were randomly collected in 30 points (15 in plantation line paired with 15 between plantation lines) in each one of the treatments SRVC, RLVC and RCVC, while in treatments TSMO, SMPC, RCAV and RCLC, given the homogeneity of the plots, samples were randomly collected in 15 points per treatment. In the former set of treatments, C storage was computed weighing values obtained in plantation line and between plantation lines according to the respective areal proportion, specific of each treatment as shown above. Therefore, statistical comparisons of soil C storage involved 15 values in all treatments. Soil samples were taken from the depths 0-5, 5-15, 15-30 and 30-60 cm. In the same depths undisturbed samples were taken using a core sampler with 100 cm³ volume for determining bulk density. Samples for soil C were air dried,

sieved (mesh size 2 mm) to determine the coarse elements content. Herbaceous biomass samples, one square meter size, were randomly collected in 15 points in each treatment.

To determine above and belowground biomass of forest species sixteen trees were observed (8 CS and 8 PM) in treatments with moderate and high soil disturbance, and selected according to average height in each plot. In slight soil disturbance treatments the forest species survival was less than 10% and so they were not accounted for in the biomass computations (Fonseca, 2005). Aboveground biomass was collected and separated in leaves, small branches (branches of year), branches and stems. To expose the root system, trenches were carefully and manually opened, and all roots were observed in their full length, collected and grouped in four diameter classes: <2 mm (very fine roots), 2-5 mm (fine roots), 5-10 mm (average size roots) and 10-20 mm (coarse roots) (Böhm 1979). All biomass samples (forest species and herbaceous vegetation) were dried at 65°C for 72 h to determine dry mass.

All plant material samples and mineral soil were analyzed for C by elemental carbon analyzer by combustion at 1,100°C and detection by Near Infrared Detector. Biomass values were converted to carbon (kg C m⁻²) by multiplying these values by the C concentration in dry matter. Soil organic carbon (SOC) contents (kg C m⁻²) were calculated by multiplying C concentration with bulk density and thickness of the mineral soil layer with a correction for coarse elements content.

The total carbon storage (TC) per unit area (kg C m⁻²) was estimated by aggregating the mean amount of C in different pools:

$$TC = C_{FS} + C_{HV} + C_{SOC}$$

Table 2. Aboveground and belowground biomass C pool in the two forest species tested (*Pseudotsuga menziesii*, *PM*, and *Castanea sativa*, *CS*, with different site preparation techniques

Treatment	Aboveground C pool (g C m ⁻²)			Belowground C pool (g C m ⁻²)			Shoot/root ratio*	
	<i>PM</i>	<i>CS</i>	Total	<i>PM</i>	<i>CS</i>	Total	<i>PM</i>	<i>CS</i>
SRVC	3.23 ^b	14.55 ^a	17.78 ^b	0.40 ^b	2.63 ^b	3.03 ^b	8.08	5.53
RLVC	3.07 ^b	13.94 ^a	17.01 ^b	0.39 ^b	2.82 ^b	3.22 ^b	7.87	4.94
RCVC	8.20 ^a	16.29 ^a	24.49 ^a	1.52 ^a	4.34 ^a	5.87 ^a	5.39	3.75
RCLC	6.75 ^a	9.10 ^b	15.85 ^b	1.26 ^a	2.40 ^b	3.66 ^b	5.36	3.79

* Values calculated with average above and belowground C pool. For the same component (above or belowground C pool) values followed by different letter in columns are statistically different ($p < 0.05$).

where C_{FS} is carbon content in above and belowground biomass of forest species; C_{HV} is carbon content in above and belowground biomass of herbaceous vegetation; C_{SOC} is carbon content in soil.

Statistical analysis comprised one-way ANOVA and multiple comparison of averages (Tukey, 5%) for assessing the effects of treatments on carbon pools. Changes of C stocks in herbaceous vegetation biomass and mineral soil under forest stands were compared with original soil.

Results and discussion

Tree C storage

As noted in materials and methods tree C storage was only determined in moderate and high site preparation intensity treatments (SRVC, RLVC, RCVC and

RCLC). The distributions of C pool at aboveground and belowground trees biomass varied significantly with species ($p < 0.000$) and treatments within the same specie ($p < 0.001$). Tree species (above and belowground biomass of *CS* and *PM* together), on average, store 20.81 g C m⁻² in SRVC, 20.23 g C m⁻² in RLVC, 30.36 g C m⁻² in RCVC and 19.51 g C m⁻² in RCLC, but always with the highest values in *CS* (Table 2 and Fig. 2). In the same order of treatments, *CS* represents 82.6, 82.9, 68.0 and 59.0% of the total C stocks in biomass of trees. The carbon sequestration in trees is proportional to the biomass increments (Madeira *et al.*, 2002; Cairns and Lasserre, 2004), so the selection of forest species to install can be an important strategy for carbon stocks (Silver *et al.*, 2000). However, Ponce-Hernández (1999) notes that, unlike most of the tropical ecosystems where carbon storage is processed mainly in biomass, in temperate and colder ecosystems the mineral soil is the main carbon reservoir.

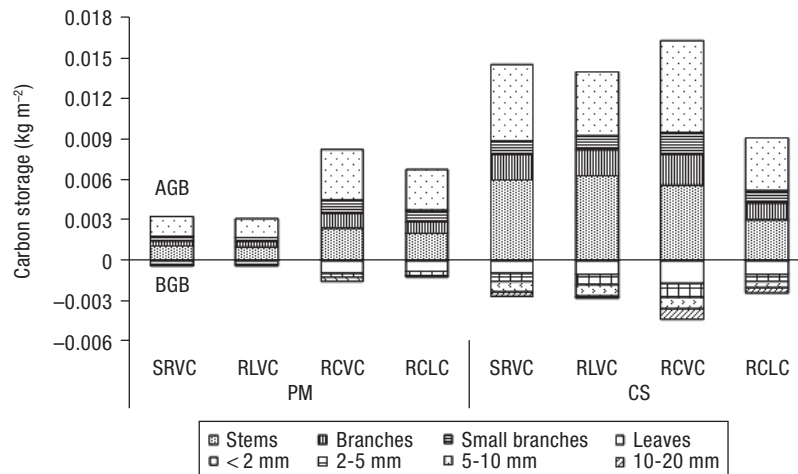


Figure 2. Carbon in aboveground (AGB by plant component) and belowground (BGB by root size class) biomass of forest species on four treatments, ranked according to soil disturbance intensity (moderate: SRVC and RLVC; high: RCVC and RCLC).

In both species the amount of C stored in above-ground biomass represented over 80% of the total C stored in trees and approximately 65% of the entire tree C was stored in stems and leaves with identical partition in both components (Fig. 2). Fine root biomass of *PM* contributed over 64% to total C storage in root biomass (root diameter below 5 mm), while in *CS* does not exceed 40% (root diameter till 20 mm). In the treatments examined here, the C distribution in above-ground biomass/belowground biomass relatively to the total C stored in the biomass, was, respectively, of 84-89%/11-16% for *PM*, and 79-84%/16-21% for *CS*. Similar proportions to those for *CS* are reported by Madeira *et al.* (2002) for young *Eucalyptus globulus* stands and Nunes *et al.* (2010) for mature *Pinus pinaster* forest, both in Portugal. Although, root biomass is present in smaller proportion, this component stores carbon for longer periods of time (Silver *et al.*, 2000). It should be noted that, in temperate forest systems, a larger accumulation of C in the aerial component is commonly found (e.g. Madeira *et al.*, 2002; Fernández-Núñez *et al.*, 2010; Nunes *et al.*, 2010). For *PM*, in general, the increased soil tillage intensities were favourable to the C storage but for *CS* the treatment with the highest soil tillage intensity (RCLC) shows the lowest value and this was due to the high mortality recorded in RCLC, nearly 50% (Fonseca *et al.*, 2011).

The shoot / root ratio for both species decreased with tillage intensity (Table 2), reflecting the improved conditions of soil for root penetration and development (Curt *et al.*, 2001; Abu-Hamdeh, 2003). Shoot/root ratio, a species-related feature, is higher in *PM* than in *CS*. In *CS* root system is deeper, with higher proportion of roots in the 10-30 cm layer and more uniformly distributed in depth, whereas in *PM* the higher root density was found in the 10-20 cm layer (Fonseca *et al.*, 2005).

Herbaceous vegetation C storage

All treatments of site preparation led to a decrease in C stored in herbaceous vegetation biomass. As expected the highest value is recorded in TSMO (0.222 kg C m⁻²) (Fig. 3). However, the contribution of shoots and roots of herbaceous vegetation is not expressive, less than 5 and 0.4% of total carbon stored in the system, respectively.

Despite the small contribution of herbaceous vegetation in the global C storage, the development of annual

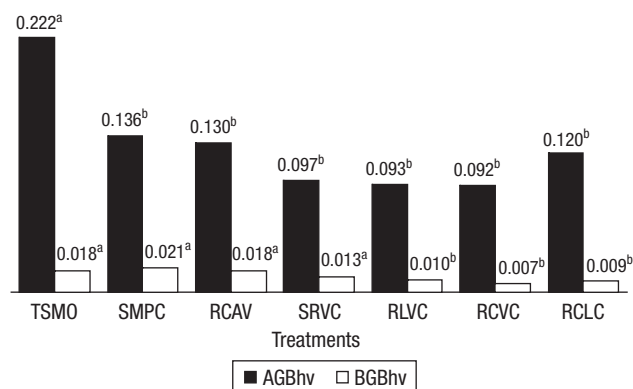


Figure 3. Carbon in above (AGBhv) and belowground (BGBhv) biomass in herbaceous vegetation on the treatments tested, ranked according to soil disturbance intensity (none: TSMO; slight: SMPC and RCAV; moderate: SRVC and RLVC; high: RCVC and RCLC). For each component (AGB or BGB) values followed by different letter in columns are statistically different ($p < 0.05$).

and perennial herbaceous plant communities played an important role in site productivity, and their rapid growth and death provided an important pool of soil organic C and nutrients (Mun and Whitford, 1998; Nicolini and Topp, 2005)

Soil C storage

Carbon stocks in the surface 60 cm of mineral soil in the various techniques of site preparation for afforestation are presented in Table 3. Overall, C stock was highest in treatments with moderate soil disturbance (SRVC, 5.19 kg C m⁻² and RLVC, 5.07 kg C m⁻²) and lowest in the high soil disturbance treatment (RCVC, 4.70 kg C m⁻² and RCLC, 3.96 kg C m⁻²). The amount of carbon in slight soil tillage treatments (SMPC, 4.89 kg C m⁻² and RCAV, 4.76 kg C m⁻²) show values similar to those of the original soil (TSMO, 4.95 kg C m⁻²), probably due to the low degree of mixing soil in the tillage affected layers, which does not favoured organic matter mineralization (Salonius, 1983). Actually, the increase in organic matter content along the soil profile is an indicator of the degree of mixing caused by soil tillage (Alcázar *et al.*, 2002), so it is reasonable to infer that when the intensity of soil disturbance increases, the mixing degree of the disturbed soil also increases. In general, C stock of both 0-5 and 5-15 cm layers of the mineral soil was significantly lower under high soil tillage intensity treatments when compared with the moderate/slight ones. Carbon accumulation in depth,

Table 3. Soil carbon storage (kg C m^{-2}) at several depths, according to treatment, expressed as mean and standard deviation

Treatment	Depth (cm)				Total soil C
	0-5	5-15	15-30	30-60	
TSMO	0.86 ± 0.10^a	1.25 ± 0.15^a	1.41 ± 0.19^a	1.33 ± 0.33^{ab}	4.85^a
SMPC	0.86 ± 0.07^{ac}	1.48 ± 0.16^a	1.46 ± 0.07^a	1.09 ± 0.10^{ab}	4.89^a
RCAV	0.86 ± 0.07^a	1.37 ± 0.08^a	1.55 ± 0.16^a	0.98 ± 0.10^b	4.76^{ab}
SRVC	0.69 ± 0.06^{ad}	1.32 ± 0.15^a	1.53 ± 0.13^a	1.65 ± 0.14^{ab}	5.19^a
RLVC	0.60 ± 0.05^{bcd}	1.20 ± 0.13^a	1.45 ± 0.13^a	1.82 ± 0.17^a	5.07^a
RCVC	0.56 ± 0.06^{bde}	1.09 ± 0.14^b	1.42 ± 0.17^a	1.63 ± 0.11^{ab}	4.70^{ab}
RCLC	0.33 ± 0.07^e	0.72 ± 0.15^b	1.33 ± 0.18^a	1.58 ± 0.17^{ab}	3.96^b

For the same depth values followed by different letter in columns are statistically different ($p < 0.05$).

mainly in the 30-60 cm layer, is clearly associated with treatment intensity of soil tillage (Table 3; Fig. 4). According to other studies (Grigal and Berguson, 1998; Post and Kwon, 2000; Balesdent *et al.*, 2000), the distribution of C within the soil profile is related to the inversion of soil layers by mechanical action and changes in physical protection of organomineral aggregates. Furthermore, organic matter mineralization and gas exchange with the atmosphere are processed faster in surface layers. Thus, the 0-5 and 5-15 cm layers in the treatments with high tillage-induced soil disturbance showed lower levels of carbon, possibly associated with the effects of increased aeration (Dick *et al.*, 1998; Schulp *et al.*, 2008), because there was a decrease of bulk density with tillage intensity (Fonseca, 2005).

Carbon accumulation was estimated as the difference between C inventories in soils with site preparation and original soil (Fig. 4). Slight soil tillage increased

carbon storage in surface layers, particularly between 5 and 15 cm (SMPC, 18.4% and RCAV, 9.6%) with decreases thereafter (−18.0 and −26.3% for SMPC and RCAV, respectively), unlike, high soil disturbance shows marked decline in the first 30 cm of soil particularly in the 0-5 cm layer (RCVC, −34.9 and RCLC, −61.6%) and increases in the 30-60 cm layer (22.6 and 18.8% for RCVC and RCLC, respectively), reaching global losses of 20% in RCLC. The latest results confirm Merino *et al.* (2004), who observed initially around 50% of C losses in soils under intensive soil tillage. Moderate soil disturbance (SRVC and RLVC) shows a different behaviour, with decreasing C in the soil profile till 5 cm (SRVC) or 15 cm (RLVC), and with gains in the 30-60 cm layer, 24.0% in SRVC and 36.8% in RLVC. Hence, changes have occurred at different rates and different depths within the soil profile. Some studies indicate soil C losses in surface mineral soil in the years immediately following trees plantation (Turner and Lambert, 2000; Paul *et al.*, 2002; Lemma *et al.*, 2006; Ordóñez *et al.*, 2008; Schulp *et al.*, 2008; Pinno and Bélanger, 2008).

In the 0-60 cm soil depth contained a total of 4.89, 4.76, 5.19, 5.07, 4.70 and 3.96 kg C m^{-2} at the SMPC, RCAV (slight soil tillage), SRVC, RLVC (moderate soil tillage), RCVC, and RCLC (high soil tillage intensity), respectively. This values show that, during the period of observations, slight and high soil disturbance were C source whilst moderate soil disturbance was C sink when compared with reference soil (TSMO, 4.95 kg C m^{-2}) (Table 3). For the same order indicated above for treatments the annual rates of release (−) or accumulation (+) of C were −0.30, −0.95, 1.20, 0.60, −1.25, and −4.95, $\text{Mg C ha}^{-1} \text{ year}^{-1}$. Thus, site preparation techniques have significant effects in changes of soil C stocks.

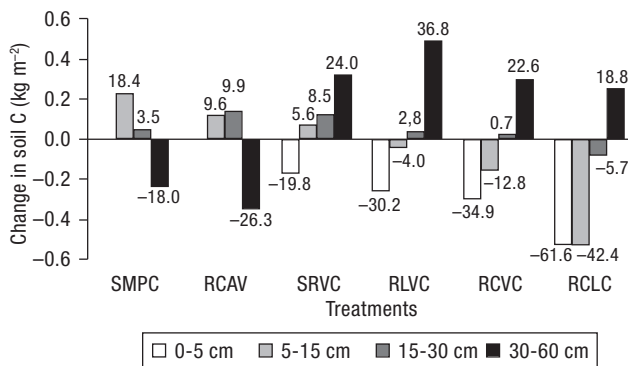


Figure 4. Variation in soil C content compared with control (TSMO), in different soil depths and on the treatments tested, ranked according to soil disturbance intensity (slight: SMPC and RCAV; moderate: SRVC and RLVC; high: RCVC and RCLC). Numbers above or below the columns indicate the percent change in soil C.

Total C storage

Site preparation techniques had effects on C stored in the tree species herbaceous vegetation and mineral soil (Fig. 5) and on the distribution of the total C stocks throughout the soil profile (Table 3). The amount of C stored in the whole system, 26 months after planting, ranges from 4.26 kg C m⁻² (RCLC) to 5.51 kg C m⁻² (SRVC) (Fig. 5). Slight (SMPC and RCAV) and moderate (SRVC and RLVC) soil disturbance treatments did not yield expressive effects on carbon storage, showing small differences from the original situation (TSMO). High soil disturbance treatments (RCVC and RCLC) contributed to a reduction in soil carbon storage, mainly in RCLC where it reaches 20% of that in TSMO.

The distribution among compartments was not the same in all treatments. For example, the treatments of moderate soil disturbance (SRVC and RLVC) proved to be more effective in adding C to the system, essentially at the expenses of forest plants. Actually, these treatments only proved to be less effective the case of in herbaceous vegetation, where their behaviour was comparable to that of high soil disturbance treatments. As found by several authors, there is a wide variation in the time period and the rate that carbon may accumulate in the soil depending on plant productivity, the physical, chemical and biological soil properties, the past history of carbon gains and the disturbances that occurred in soil (Post and Kwon, 2000; Silver *et al.*, 2000; Paul *et al.*, 2002), as shown by results presented in this study.

Soil was the main carbon compartment of total carbon storage, representing over 90% in all treatments,

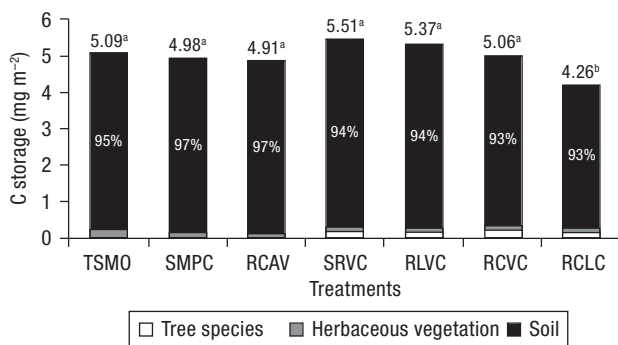


Figure 5. Carbon distribution in the system's compartments, on the treatments ranked according to soil disturbance intensity (none: TSMO; slight: SMPC and RCAV; moderate: SRVC and RLVC; high: RCVC and RCLC). Numbers above columns represent total carbon storage; numbers inside columns indicate percent of total C stored in soil. For treatments, averages with the same letter are not significantly different ($p < 0.05$).

reaching values greater than 95% in the original soil (TSMO) and in those with slight soil tillage intensity (SMPC and RCAV). The high soil disturbance treatments (RCLC) caused a stronger decrease of soil C, which was also noted by Merino *et al.* (2004). Temporary losses of soil C can follow plantation establishment, depending on site preparation techniques (Johnson, 1992; Balesdent *et al.*, 2000; Paul *et al.*, 2002).

As soil is the largest C pool in all treatments, it is important to understand the effects of site preparation techniques on C inputs and losses to the soil. Twenty six months after stand installation, only two of the six treatments examined here were C sinks, SRVC (194 g C m⁻² year⁻¹) and RLVC (129 g C m⁻² year⁻¹). Therefore, as these results clearly stress, C accumulation is one potential added benefit of moderate soil tillage in Mediterranean climate.

Conclusions

After 26 months since forest mixed stand installation in a Mediterranean region, more than 90% of the total carbon stored in the whole system was found in the soil, and more than 60% was stored in the 0-30 cm soil layer, for an average amount, down to 60 cm depth, of around 5 kg C m⁻² in the whole experimental area. Besides the short range of C amounts found in the set of site preparation techniques under test, either in absolute terms or in their relative contribution to C stored by the forest stand, soil C total amounts decreased as tillage intensity increased, with statistical significance in the case of the treatment inducing highest soil disturbance. Therefore, high intensity site preparation techniques, at this stage of stand development studied, have negative impacts on soil C pool. The site preparation techniques most effective in accumulating C in the 0-60 cm layer were those inducing moderate soil disturbance, where the soil was a C sink with gains, in average, of near 1 Mg C ha⁻¹ in more than two years.

The uppermost 5 cm of soil are those with least C amount, which significantly declined as soil disturbance intensity increased. The increase of C amount with soil depth was also affected by tillage intensity, being higher in those treatments with higher soil disturbance due to site preparation. Site preparation techniques affected C distribution in the soil profile, reflecting the reversion of soil layers, increasingly more evident as tillage intensity increases, in depth and in surface area.

As a young forest stand, tree species had a small contribution to total C storage (around 5%). The quantity of carbon per unit area was higher in *Castanea sativa* (CS) than in *Pseudotsuga menziesii* (PM), and most of it, in both species, was stored in the above ground biomass, but with higher shoot/root ratio on PM than in CS. In both species, shoot/root ratios decreased from moderate to high tillage intensity treatments, reflecting the positive effect of deeper soil tillage operations on root expansion conditions.

The contribution of above-ground biomass and roots of herbaceous vegetation to total C stored in the system was very low as expected from the vegetation clearance provided by soil preparation techniques.

The global carbon storage in the forest stand was affected by soil disturbance induced by site preparation techniques. The higher C amounts were found where moderate intensity techniques were applied, and decreased in the high intensity treatments.

Results presented emphasize not only the importance of the soil pool for carbon storage in the early stages of stand development but also increases awareness to the need of selecting appropriate site preparation techniques for stand installation able to reduce the disturbance related impacts on soil and system C storage.

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